

US008952138B2

(12) United States Patent

Shultz et al.

(10) **Patent No.:**

US 8,952,138 B2

(45) **Date of Patent:** *Feb.

*Feb. 10, 2015

(54) REFOLDING PROTEINS USING A CHEMICALLY CONTROLLED REDOX STATE

(75) Inventors: **Joseph Edward Shultz**, Santa Rosa

Valley, CA (US); Roger Hart, Loveland, CO (US); Ronald Nixon Keener, III,

Newbury Park, CA (US)

(73) Assignee: Amgen Inc., Thousand Oaks, CA (US)

(*) Notice: Subject to any disclaimer, the term of this

patent is extended or adjusted under 35

U.S.C. 154(b) by 403 days.

This patent is subject to a terminal dis-

claimer.

(21) Appl. No.: 12/820,087

(22) Filed: Jun. 21, 2010

(65) **Prior Publication Data**

US 2010/0324269 A1 Dec. 23, 2010

Related U.S. Application Data

- (60) Provisional application No. 61/219,257, filed on Jun. 22, 2009.
- (51) Int. Cl. C07K 1/22 (2006.01) C07K 1/113 (2006.01)

(58) Field of Classification Search None

See application file for complete search history.

(56) References Cited

U.S. PATENT DOCUMENTS

6,660,843	B1	12/2003	Feige et al.
6,808,902		10/2004	Treuheit et al.
7,138,370	B2	11/2006	Oliner et al.
7,511,012		3/2009	Han et al.
7,723,490		5/2010	Treuheit et al.
2008/0214795		9/2008	Ramanan et al.
2010/0267936	A1	10/2010	Treuheit et al.

FOREIGN PATENT DOCUMENTS

EP	1845103 A1	10/2007
WO	92/04382 A1	3/1992
WO	99/42486 A1	8/1999

OTHER PUBLICATIONS

Cowley, D.J, & Mackin, R.B., "Expression, purification and characterization of recombinant human proinsulin," FEBS Lett 402:125-130 (1997).

De Bernardez Clark, Eliana, et al. "Oxidative Renaturation of Hen Egg-White Lysozyme, Folding vs aggregation." Biotechnol, Prog. 14: 47-57 (1998).

Rudolph & Lilie, "In vitro folding of inclusion body proteins," FASEB J. 10:49-56 (1996).

Creighton, T.E., "Renaturation of the reduced bovine pancreatic trypsin inhibitor," J. Mol. Biol. 87:563-577 (1974).

Stöckel, Johannes, et al., "Pathway of detergent-mediated and peptide ligand-mediated refolding of heterodimeric class II major histocompatibility complex (MHC) molecules," Eur J Biochem 248:684-691 (1997).

St John et al., "High pressure refolding of recombinant human growth hormone from insoluble aggregates. Structural transformations, kinetic barriers, and energetics," J. Biol. Chem. 276(50):46856-63 (2001).

Lilie, Schwarz & Rudolph, "Advances in refolding of proteins roduced in *E. coli*," Current Opinion in Biotechnology 9 (5):497-501 (1998).

Tran-Moseman, Schauer & Clark, "Renaturation of Escherichla coli—Derived Recombinant Human Macrophage Colony-Stimulating Factor," Protein Expression & Purification 16(1):181-189 (1999). Darby, N.J., et al., "Refolding of Bovine Pancreatic Trypsin InhibitorviaNon-native Disulphide Intermediate," J Molecular Biol. 249(2):463-477 (1995).

Wang, Xi-De, "Perturbation of the antigen-binding site and staphylococcal protein A-binding site of IgG before significant changes in global conformation during denaturation: an equilibrium study,"Biochem. Prog. 14; 47-54 (1998).

Singh el al., "Solubilization and Refolding of Bacterial Inclusion Body Proteins," J. Bioscience and Bioengineering, vol. 99(4), pp. 303-310 (2005).

DeBernadez Clark, Eliana, "Refolding of recombinant proteins," Current Opinion in Biotechnology, vol. 9 p. 157-163 (1998).

DuBernardez Clark, Eliana, "Protein Refolding for industrial processes," Current Opinion in Biotechnology, vol. 12, pp. 202-207 (2001).

Javaherian, K. et al., "Laminin Modulates Morphogenic Properties of the Collagen XVIII Endostatin Domain," *J. Biol. Chem.* 277(47):45211-45218, Nov. 22, 2002.

Primary Examiner — Yunsoo Kim (74) Attorney, Agent, or Firm — David B. Ran

(57) ABSTRACT

A method of refolding proteins expressed in non-mammalian cells present in concentrations of 2.0 g/L or higher is disclosed. The method comprises identifying the thiol pair ratio and the redox buffer strength to achieve conditions under which efficient folding at concentrations of 2.0 g/L or higher is achieved and can be employed over a range of volumes, including commercial scale.

24 Claims, 8 Drawing Sheets